Reference: *Biol. Bull.*, 140: 274–283. (April, 1971)

REGULATION OF GONAD DEVELOPMENT IN THE BAY SCALLOP, *AEQUIPECTEN IRRADIANS* LAMARCK[1]

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Gonad development in the annual reproductive cycle of temperate climatic zone marine invertebrates is correlated with seasonal changes in the environment. Gonad development has been induced in molluscs (Loosanoff and Davis, 1950; Sastry, 1963) and barnacles (Crisp, 1957; Patel and Crisp, 1960) outside the normal breeding period by maintaining the animals at the appropriate temperatures and providing food. The effect of photoperiod on maturation and breeding of *Balanus* has been reported by Barnes (1963). The influence of food on gonad development in sea urchins and scallops has been reported by Giese (1959) and Sastry (1966). Barnes (1967) found that interruption of food supply leads to regression of ovarian tissue in barnacles, especially in the early stages of development. Although environmental influence on gonad growth and gametogenesis is apparent, the mechanism of control is still unclear.

Sastry (1968) reported that gonad growth and gametogenesis are initiated in bay scallops, *Aequipecten irradians* Lamarck, when the animals are exposed to a minimum threshold temperature with food. In the absence of food or when the temperature remains below the threshold level, the oogonia develop but oocyte growth does not occur. This suggests that nutrient reserves from the ingested food are utilized during gonad growth and gametogenesis. The nutrient reserves are apparently transferred from the storage organ, the digestive gland, to the gonad and utilized by the developing gametes for synthesis of various biochemical constituents.

The present paper considers the influence of temperature on the transfer of nutrient reserves.

Materials and Methods

Source and maintenance of animals

Bay scallops collected during the last week of November from Buzzards Bay, Massachusetts (10° C and 32‰), were obtained from the Supply Department, Marine Biological Laboratory, Woods Hole. The scallops were maintained in the laboratory at 5° C and 15° C in recirculating sea water aquaria (Dayno Aqua-Labs, Inc., Lynn, Massachusetts) with a photoperiod of 12 hours light and 12 hours darkness. Scallops were fed daily on naked flagellates, *Monochrysis lutheri* Droop cultured in the laboratory on an enriched sea water medium described by Davis and Guillard (1958). Three liters of food organisms (2–3 million cells/ml) were provided daily to a group of 80 scallops held at each experimental temperature.

[1] This study was supported by grants from the National Science Foundation, Sea Grant College Program, University of Rhode Island, Kingston, Rhode Island 02881.

Assay of reproductive activity

At the beginning of the experiments, ten scallops were sacrificed, and the gonad index (gonad weight/body weight × 100), digestive gland index (digestive gland weight/body weight × 100), and the stage in gametogenesis were determined (Sastry, 1966). Five animals were removed at intervals from each experimental temperature and the same measurements made. The oocyte diameter in each animal from each sample was measured with an ocular micrometer.

Radiotracer experiments

Radiotracer experiments were performed by injecting uniformly labelled ^{14}C-leucine (New England Nuclear Corp., Boston, Massachusetts) into the digestive gland of the scallops. Radioactive leucine (specific activity of 240 MC/m*M* dis-

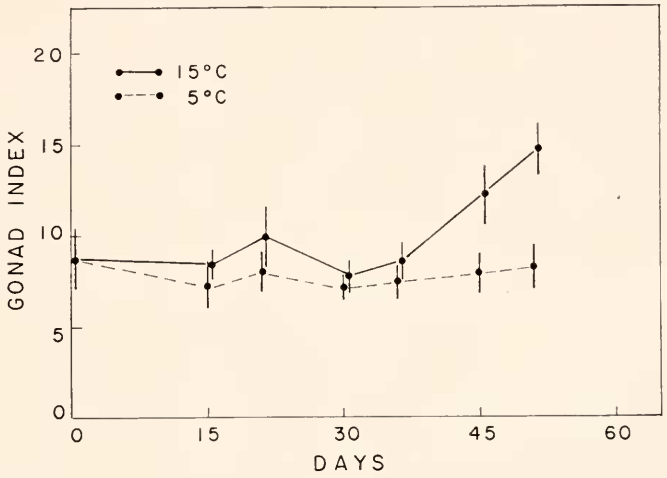

FIGURE 1. Gonad growth response of scallops at 5° C and 15° C.

solved in 0.5 ml. 0.01 *N* HCl was added to 0.5 ml of sea water (32‰) to make an approximately isotonic solution. Ten μl (0.5 μC) of solution per animal was injected into scallops held at 5° C and 15° C on the 15th, 30th and 45th day after the beginning of the experiment. The scallop injected with the labelled amino acid were placed in battery jars containing sea water of equilibrated temperature and kept in contsant temperature incubators. Each scallop was fed 100 ml of food organisms a day. Four or five animals were sacrificed after one day and again after one week to determine the gonad and digestive gland indexes and the stage in gametogenesis. Pieces of body tissues weighing 15–20 mg taken from each animal were dissolved in 1 ml of Soluene-100 (Packard Instrument Co.) in low potassium vials. The vials were kept for at least 18 hours at 35° C to aid in dissolving the tissue. The vials were then filled with Bray's solution (Bray, 1960). The scintillation mixture contained 60 g naphthalene, 4 g PPO, 500 ml POPOP, 100 ml methanol, 20 ml of ethylene glycol in 880 ml of p-dioxine to make up to

a total volume of 1 liter. Radioactivity was measured with a Packard Tri-Carb liquid scintillation counter (model 3380) attached to a computer print out. Appropriate corrections were made for background and quenching.

The total disintegrations per minute (DPM) for organs of each animal were calculated by the following method:

$$\text{Total DPM/organ} = \text{DPM/mg tissue} \times \text{total weight of organ (mg)}.$$

The DPM for all body organs were added to obtain the total DPM per animal. Incorporation of labelled amino acid into the organs is calculated by the following method:

$$\text{Radioactivity index} = (\text{Total DPM/organ} \times 100/\text{Total DPM/animal})$$

Statistical analysis of the data was made with the aid of IBM-360 computer.

Results

Gonad growth response of scallops at 5° C and 15° C

The mean gonad index values of scallops held at 5° C and 15° C are shown in Figure 1. The gonad index remained approximately the same for all samples from 5° C. The scallops at 15° C showed an increase of gonad index following day 30. The differences between the gonad indexes of samples kept at the two temperatures became significant by day 45.

The gonad and digestive gland indexes of scallops show a reciprocal relationship during the reproductive cycle (Sastry, 1970a). The digestive gland index, greater than the gonad index during the vegetative and resting periods, decreases with the gonad growth. This decrease has been attributed to the transfer of nutrients to the gonads (Sastry, 1966, 1968). The relationship between the two organ indexes of scallops exposed to 5° C and 15° C is shown in Figure 2 and Table I. The digestive gland index remained near a constant level above the gonad index for scallops at 5° C. At 15° C, however, the digestive gland index fluctuated, and showed an increase with the growth of gonads. Apparently the clear reciprocal relationship observed for field population does not apply to animals held in the laboratory with abundant food.

Gametogenesis in scallops at 5° C and 15° C

The scallops examined at the beginning of the experimental period had only primary germ cells. The oocyte growth in scallops held at 5° C and 15° C is shown in Figure 3. Oogonia developed at 5° C but there was no oocyte development. The scallops held at 15° C developed oocytes in the cytoplasmic growth phase by day 21 and the vitellogenesis growth phase by day 36. Most oocytes were in the vitellogenesis growth phase on day 51.

Spermatocytes and spermatids were observed in the scallops at the beginning of the experiment. In the scallops held at 5° C spermatocytes were the dominant stage throughout the experimental period, but a few spermatozoa also were observed. At 15° C spermatozoa were predominant, but spermatocytes and spermatids were present.

Incorporation of ^{14}C-leucine into the digestive gland and gonad of scallops at 5° C and 15° C, 24 hours after injection

The mean radioactivity index for ^{14}C-leucine incorporation into the digestive gland and the gonad of scallops at 5° C and 15° C is given in Figure 4 and Table II. The animals injected with the labelled amino acid on day 15 and 30 at 5° C showed a wide variation of incorporation into the digestive gland. The mean radioactivity index for labelled amino acid incorporation was slightly higher in the

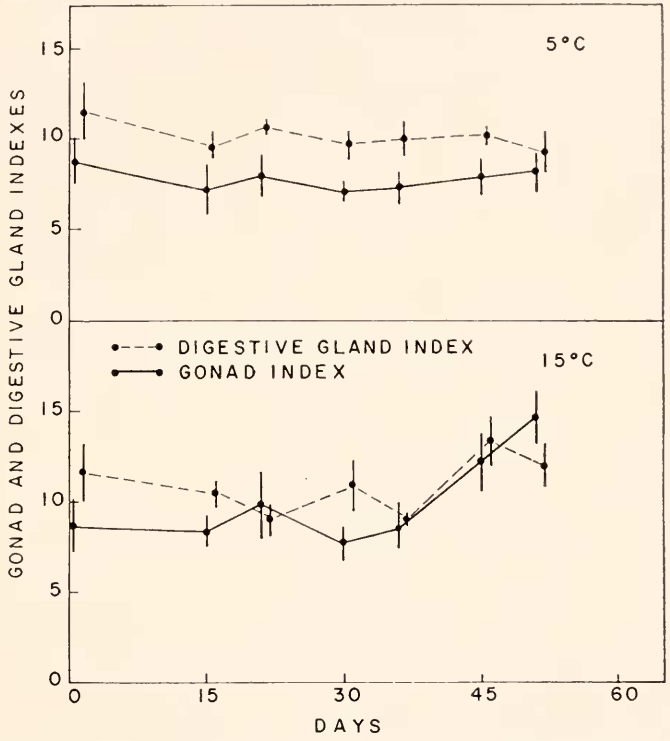

FIGURE 2. Mean gonad index and digestive gland index of scallops exposed to temperatures of 5° C and 15° C.

sample injected on day 30. Incorporation of ^{14}C-leucine into gonads was much less than into the digestive gland in all the samples. The gonads of samples injected on days 30 and 45 took up slightly more than those injected on day 15.

In the scallops held at 15° C the incorporation of ^{14}C-leucine into the digestive gland of all samples was about the same except for day 30, when it was slightly higher. Again the gonads took up much less amino acid than the digestive gland. The mean radioactivity index for incorporation of ^{14}C-leucine into the gonads was about the same in samples injected at different time intervals.

Incorporation of ^{14}C-leucine into both digestive gland and gonad of scallops held at 15° C was higher than in the animals at 5° C (Table II).

Incorporation of ^{14}C-leucine into digestive gland and gonad of scallops at 5° C and 15° C, one week after injection

The incorporation of ^{14}C-leucine into the digestive gland and gonad of scallops at 5° C and 15° C is shown in Figure 4 and Table III. Uptake of labelled amino acid by the digestive gland was about the same on day 15 and day 45 for scallops held at 5° C. The incorporation of ^{14}C-leucine into the gonad, while much lower than that for the digestive gland, was slightly higher on day 45 than on day 15.

In the scallops held at 15° C, the mean radioactivity index for incorporation of labelled amino acid into the digestive gland was about the same in all the samples. At this temperature, however, the gonads incorporated more ^{14}C-leucine than the digestive gland on days 30 and 45.

Table I

Mean gonad and digestive gland indexes of scallops at 5° C and 15° C

Day	Organ	5° C	15° C	Significance (t-test) t
0	G	8.7 ± 1.4	8.7 ± 1.4	—
	D	11.6 ± 1.6	11.6 ± 1.6	—
15	G	7.3 ± 1.3	8.4 ± 0.8	1.58
	D	9.6 ± 0.8	10.4 ± 0.7	1.74
21	G	8.0 ± 1.1	9.8 ± 1.8	1.87
	D	10.7 ± 0.3	9.0 ± 0.8	4.41*
30	G	7.2 ± 0.6	7.7 ± 0.9	1.08
	D	9.6 ± 0.8	10.9 ± 1.4	1.08
36	G	7.4 ± 0.9	8.6 ± 1.1	2.10
	D	10.1 ± 0.8	9.0 ± 0.1	1.95
45	G	7.9 ± 1.0	12.2 ± 1.6	5.05*
	D	10.3 ± 0.4	13.4 ± 1.4	4.64*
51	G	8.3 ± 1.1	14.7 ± 1.4	8.13*
	D	9.4 ± 1.1	12.1 ± 1.2	3.91*

G, Gonad; D, Digestive gland; * $P \leq 0.05$.

The difference in the uptake of ^{14}C-leucine into the gonads of scallops held at 5° C and 15° C is statistically significant (Table III). Incorporation of ^{14}C-leucine into the digestive gland of scallops held at 15° C was higher than in the animals held at 5° C. The difference is only significant in the samples injected on day 45 ($P < 0.05$).

Discussion

The influence of environmental factors on the gonad development of marine invertebrates has been demonstrated, but the mechanisms controlling gonad growth in the reproductive cycle are not yet clear. Breeding condition has been induced in

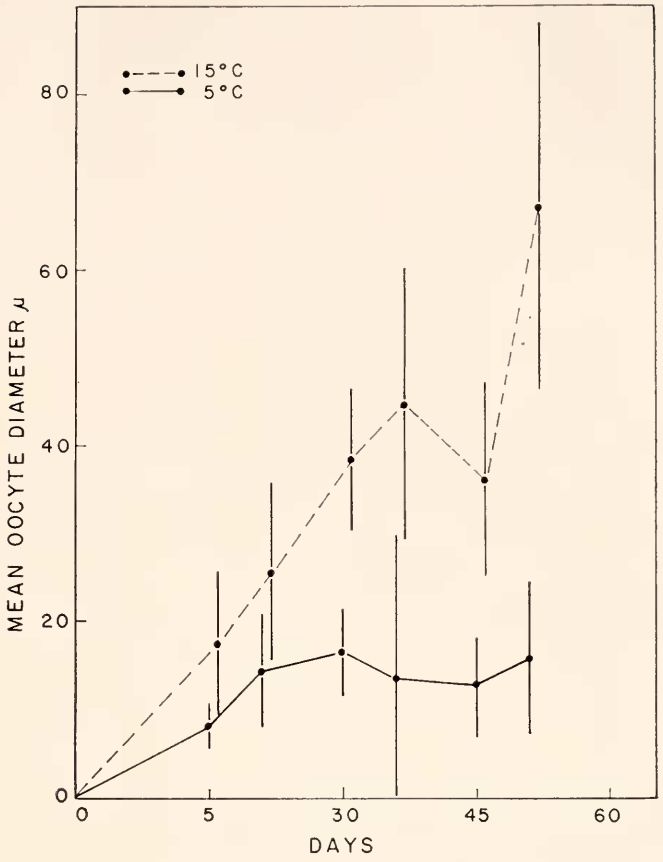

FIGURE 3. Oocyte growth in scallops at 5° C and 15° C.

TABLE II

Incorporation of ^{14}C-leucine into gonad and digestive gland of scallops at 5° C and 15° C, 24 hours after injection

Day of injection	Organ	5° C Radioactivity index	15° C Radioactivity index	Significance (t-test) t
15	G	3.8 ± 2.3	11.1 ± 7.8	1.99
	D	13.6 ± 10.4	18.9 ± 4.4	0.96
30	G	6.8 ± 3.8	7.3 ± 3.4	0.23
	D	20.7 ± 17.7	26.7 ± 10.0	0.66
45	G	7.0 ± 2.0	9.8 ± 1.4	2.51*
	D	14.0 ± 2.5	19.1 ± 2.5	3.00*

G, Gonad; D, digestive gland; * $P \leq 0.05$.

bivalve molluscs and barnacles by maintaining the animals at appropriate temperatures in the winter and supplying food. This suggests a temperature and nutritional influence on gonad development (Loosanoff and Davis, 1950; Patel and Crisp, 1960; Sastry, 1963). Crisp (1957) reported that *Balanus* required maintenance at colder temperatures for a prolonged period before they could be induced to breed at warm temperatures. However, exposure to warm temperatures alone failed to induce breeding condition in *Balanus*. Starvation of a number of species

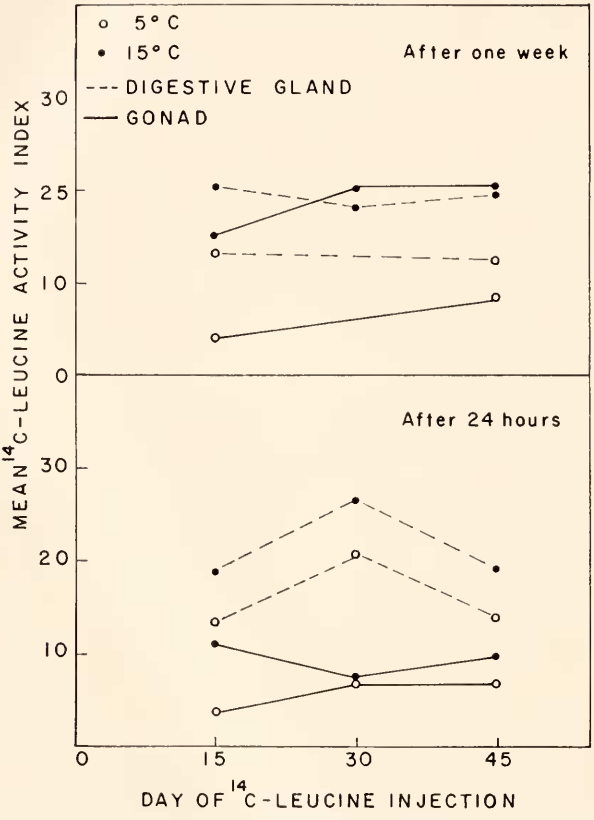

FIGURE 4. Incorporation of ^{14}C-leucine into gonad and digestive gland of scallops, 24 hours after injection and one week after injection.

at the beginning of the reproductive period was reported to prevent gonad growth which suggests that food influences gonad development (Giese, 1959; Sastry, 1966; Barnes, 1967). Apparently, the nutrient reserves from the ingested food must be transferred to gonads for growth to occur and these materials are utilized there in the biochemical synthesis of the developing gametes (Giese, 1959; Barnes, Barnes and Finlayson, 1963). It has been suggested that nutrient reserves are transferred from the storage organ in barnacles and starfish or directly from gut to perivisceral fluid and then to gonads in sea urchins without significant storage (Giese, 1959;

Farmanfarmaian and Phillips, 1962; Barnes, Barnes and Finlayson, 1963; Mauzey, 1966). The transfer of nutrient reserves to the gonads is thought to occur with the initiation of gonad development by establishment of a gradient between source and utilization (Barnes, Barnes and Finlayson, 1963). However, there are only a few studies examining the distribution and utilization of nutrients during reproduction in marine invertebrates. Allen (1962) found that *Mya arenaria* fed with ^{32}P labelled *Pheodactylum* assimilates greatest concentrations of ^{32}P in the digestive gland with little evidence of rapid build up in the ovary. In *Calanus,* however, Marshall and Orr (1955, 1961) found that 70% of the ^{32}P assimilated by the female copepod passes rapidly into the ova. The results of the present study show that transfer of nutrient reserves to gonads occurs in scallops with the initiation of gonad development. Gonad development is initiated in fed animals maintained within a range of temperatures (Sastry, 1968). Under these conditions, the nutrient reserves are apparently transferred to the gonads. Temperature below the

TABLE III

Incorporation of ^{14}C-leucine into gonad and digestive gland of scallops at 5° C and 15° C one week after injection

Day of injection	Organ	5° C Radioactivity index	15° C Radioactivity index	Significance (t-test) t
15	G	4.2 ± 1.1	15.7 ± 7.8	3.12*
	D	13.2 ± 12.9	20.6 ± 11.8	0.96
30	G	—	20.0 ± 12.0	—
	D	—	18.2 ± 1.8	—
45	G	8.5 ± 2.2	20.4 ± 3.4	6.54*
	D	12.4 ± 3.4	19.8 ± 3.0	3.48*

G, Gonad; D, Digestive gland; * $P \leq 0.05$.

required minimum supresses gonad growth and the nutrient reserves are accumulated in the digestive gland. The nutrient reserves stored in the digestive gland may supplement the nutritional intake needed for gonad growth. However, the animals maintained at temperatures exceeding a certain maximum also fail to develop gonads. Perhaps this is due to increased metabolic utilization or failure to regulate the synthetic processes of the developing gametes.

Gametogenesis occurs simultaneously with gonad growth in scallops. Oocytes enter the cytoplasmic growth phase when the animals are exposed to the required minimum temperature with food. Either subthreshold temperature or starvation will prevent the oocytes from developing (Sastry, 1968). Apparently initiation of the cytoplasmic growth phase is regulated by the transfer of nutrients to the gonads, this transfer, of course, being dependent on the temperature. These nutrients are obviously utilized by the oocytes during their growth to mature eggs. The nutrient transfer to the gonads is therefore not only regulated by environmental factors, but also by the nutritional needs of the developing oocytes.

The significant increase in the incorporation of ^{14}C-leucine into gonads of scallops with oocytes in the cytoplasmic growth phase at 15° C further suggests

that the rate of nutrient transport to the gonads is regulated by the temperature and the stage of oocyte development (Figs. 3 and 4). However, the uptake of labelled amino acid into gonads of scallops at 5° C and 15° C measured 24 hours after injection is not significantly different. The nutrient transfer may occur slowly at a rate regulated by the developing oocytes.

The difference in the incorporation of ^{14}C-leucine into the gonads of scallops at 5° C and 15° C could have been influenced by the temperature, or the stage of oocyte development, or both. Incorporation of ^{14}C-leucine into gonads of scallops with oogonia at 5° C is much less than those with oocytes in the cytoplasmic growth phase at 15° C, especially one week after injection. Incorporation of ^{14}C-leucine has been shown to be involved in protein synthesis (Raven, 1961; Williams, 1965). It has also been reported that protein synthesis occurs in growing medium-sized oocytes but is absent in very young or mature unfertilized eggs of the sea urchin. It is possible that oocytes entering the growth phase stimulate gonad development, with the rate of gonad development depending on the rate at which nutrient reserves are utilized by the growing oocytes for synthesis of various biochemical constituents. Oocytes entering the cytoplasmic growth phase may begin protein synthesis by utilizing the nutrients taken up from the digestive gland. The cytoplasmic growth phase of oocytes may be triggered either by direct action of temperature or by neurosecretion or both (Lubet, 1966; Sastry, 1970b).

It is a pleasure to thank Dr. Eugene Jackim and Dr. Earl Davey, National Marine Water Quality Laboratory, West Kingston, for the use of facilities for radioactivity measurements and for assistance during this study. Thanks are also due to Miss Katherine Saunders for technical assistance.

Summary

1. Gonad growth and oocyte cytoplasmic growth were induced in scallops exposed to 15° C with food. At 5° C only oogonia developed.

2. Scallops injected with ^{14}C-leucine and measured 24 hours after injection showed greater incorporation of labelled amino acid into both digestive gland and gonad at 15° C than at 5° C, but the difference in uptake between the two groups was not significant.

3. Incorporation of ^{14}C-leucine into gonads of scallops measured one week after injection was significantly higher at 15° C than at 5° C.

4. The gonad growth and oocyte growth of scallops is controlled by food and temperature. Both external environmental factors and the stage of oocyte development seem to regulate nutrient transfer to gonads and therefore control growth activity.

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